SEQUELAE OF THE LD/50 X-RAY EXPOSURE OF THE PRE-IMPLANTATION MOUSE EMBRYO: DAYS 0.0 TO 5.0[1]

R. RUGH, L. DUHAMEL, C. SOMOGYI, A. CHANDLER, W. R. COOPER, R. SMITH AND G. STANFORD

Radiological Research Laboratory, College of Physicians and Surgeons, Columbia University, New York, N. Y. 10032

In a recent study by Rugh and Wohlfromm in 1963 it was found that it took varying exposures of x-rays to kill *in utero* half of the mouse embryos of different ages, and in a still further study by the same investigators in 1965, pre-natal exposures and post-natal mortality data were presented. It was impossible to establish the LD/50/30 of x-rays for the mouse embryos of the first 5 days of gestation (since they were either killed *in utero* or appeared to be unaffected). It was therefore decided to use the LD/50 dose for the embryos *in utero* and study the sequelae exhibited by their survivors. In other words, doses from 100 r to 350 r were used which killed approximately half of the early mouse embryos *in utero,* and those which survived this radiation insult were examined during their lifetime for evidence of permanent but tolerable damage.

In a previous study by Rugh, Duhamel, Chandler and Varma in 1964 it had been shown that when the mouse embryos were exposed at the various gestation ages to the uniform dose of 100 r x-rays, there was variable response with respect to cataractogenesis. The variations in response were related to the gestation age at exposure. It was found that the highest incidence of cataract development, at 18 months of age, occurred when the mice were x-rayed at the time of fertilization or 0.0 days. Such mice developed 97% cataracts while the parallel controls showed 17% for the females and 13% for the males. The difference between those x-rayed and the controls represented at least 80%, which could be considered the incidence of radiation-induced cataracts. There was also a rather high incidence of cataracts among those x-irradiated during the next several days of gestation, so that it seemed in order to investigate this matter further.

This study includes effects of the LD/50 x-ray exposure of early mouse embryos in terms of litter size; sex ratios at birth and at 30 days; monthly weight variations; life span, blood, and skeletal changes and the etiology of cataracts.

Materials and Method

White female mice of the CF1 strain were put through a single pregnancy, using males of the same strain for mating, prior to their use in this experiment. For the experimental pregnancies the females were time-mated for two hours (8–10 AM) and those with vaginal plugs were segregated and marked as to the time of

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conception. Some were x-rayed immediately (0.0 time) and others on the various days from 0.0 to 5.0 at which time implantation is in progress. Implantation actually begins at 4½days but is a continuous process for several days prior to placenta formation. The dose of x-rays varied with the gestation age, being based upon a prior study (Rugh and Wohlfromm, 1963) in which the dose which would kill half of the embryos was determined. These doses are given in Table I in connection with litter size and sex ratios.

Upon delivery the mice were counted, sexed, anomalies recorded, and all were given to foster mothers who had not been x-rayed but who had had simultaneous litters. These foster mothers therefore provided normal post-natal care and nutrition until the time of weaning.

Those mice surviving at one month constituted the initial group for the selection of mice for this study. One hundred controls and 50 experimentals (half males and

TABLE I

This table gives the results of exposing early mouse embryos to the previously-determined LD/50 dose of x-rays, measured in terms of average litter size, sex ratios, and the survival of the respective sexes during their first 30 days of life. It demonstrates a drastic reduction in initial litter size, and (with the exception of gestation day 3 exposed to 140 r) a manifold increase in lethality during the first 30 post-natal days

Gestation age	LD/50 x-rays	Litter size average	Offspring at birth		Percentage lost in 30 days		
			Males	Females	Males	Females	Total
Controls	00	10.3	66	78	3.3%	3.8%	3.5%
0.0 gest. days	100 r	4.6	41	37	17.1	13.5	15.4
0.5 gest. days	275 r	6.7	45	48	11.1	12.5	11.8
1.0 gest. days	350 r	7.6	56	50	19.6	24.0	21.7
2.0 gest. days	125 r	6.1	44	42	11.5	9.5	10.4
3.0 gest. days	140 r	5.9	40	55	2.5	3.6	3.1
4.0 gest. days	330 r	6.5	48	73	37.5	13.7	23.1
5.0 gest. days	350 r	8.1	70	69	20.0	14.5	17.9
Totals (x-rayed)			344	374	17.0%	13.0%	14.8%

half females) were selected at random from those x-irradiated on each gestation day. Each mouse was earmarked for permanent identification and was examined and weighed each month through 31 months, or until none was left. At 2, 6, 9, 12, 18 and 24 months the eyes of every mouse in the series (2176 eyes) were examined by practicing ophthalmologists of this institution. The slit lamp was used to determine whether there were corneal opacities or incipient nuclear or cortical cataracts in the process of development. The pupils of the eyes were dilated at least one-half hour prior to the examination by a drop of 2.5% isopto-homatropine, used as a mydriatic. Thus, with each mouse identified by number and the condition of each eye recorded, it was possible to follow the onset and the development of cataracts in this study.

The x-ray facilities consisted of parallel tubes in cross fire spaced at 72 cm. each from the center of the gravid uterus. The machine was run at 184 KVP,

30 MA, with filters of 0.28 Cu and 0.50 Al, and having an HVL of 0.6 mm. Cu. The dose rate was 50 r/minute. The absorption from the plastic container and the scattering of radiations from the bodies of the mice balanced out so that the estimated dose absorbed by the embryos was very close to the air dose calculated at the position of the embryos described above.

Experimental Data

The litter size at birth gives a fair indication as to the survival of embryos exposed to various doses and the several gestational ages. However, an accurate lethal

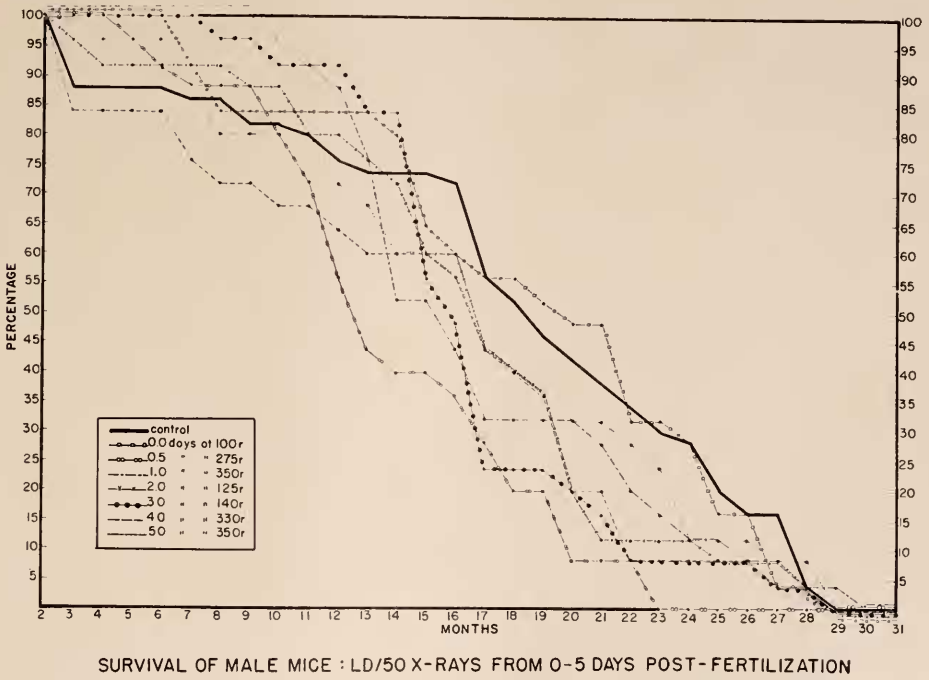


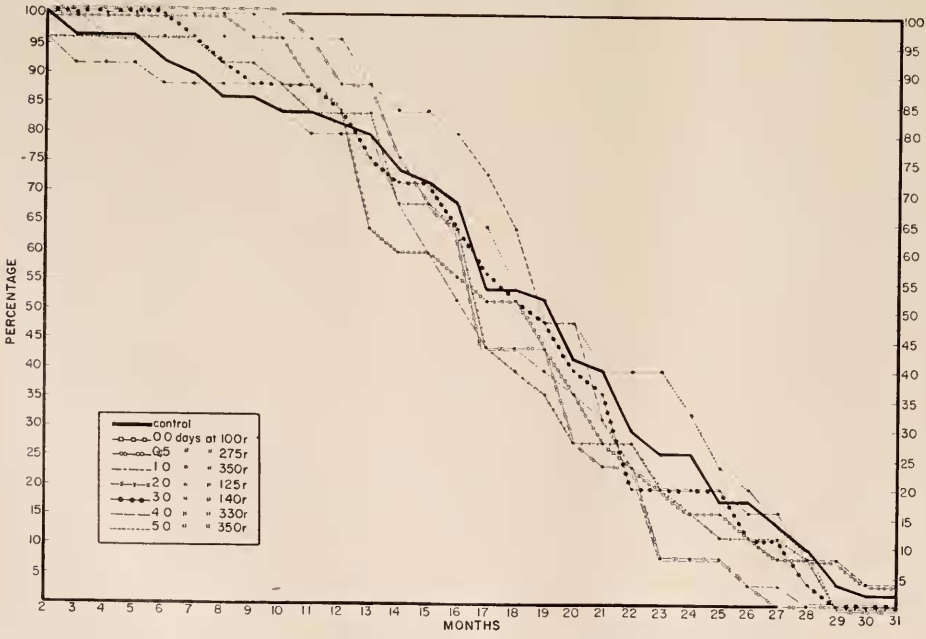
Figure 1. The dose of x-rays delivered to male mice on days designated varied because of the previous determination of the lethal dose to approximatly half of such embryos. Nevertheless, the survival curve over the 31-month period did not vary much with the different gestational age exposures, although most were depressed slightly below the solid control line of survival. The most sensitive gestational age was 0.5 day after insemination, at which time early embryos received 275 r x-rays.

x-ray dose to half of the embryos (LD/50) for pre-implantation stages is rather difficult to establish without determining the number of viable eggs fertilized. This cannot be done without sacrifice of the animal. The average implantation number for this strain of mice was found to be between 11 and 12.

Among those mice which came to term there was a slightly higher number of females than of males, and during the first 30 days of their lives 17% of the males and 13% of the females died. In all cases except those x-rayed at 3.0 days to 140 r,

deaths during these first 30 days of life far exceeded the record for the controls, which was 3.5%. Since this study was based upon x-rayed mice which survived embryonic and fetal life, and also the first 30 days of post-natal life, and were studied throughout their life span, the data for weight, skeletal, blood, and cataract changes relate only to the hardiest of the mice exposed *in utero*. It must therefore be presumed that death of many mice deprived us of further statistical data relating to these physical variables.

The survival of mice selected at one month of age is shown for males and females separately in Figures 1 and 2 during the succeeding 30 months. Note the LD/50 exposures for the various gestation days which kill half of the embryos in utero. These figures (1 and 2) suggest that there is little permanent damage, in terms of life-shortening, when the experimental mice are compared with the controls. The controls are shown in heavy solid lines and those of the various gestation ages receiving different exposures are shown in the other curves. The experimental males did show a slight reduction in survival value, while the difference between the sexes was not at all pronounced in the controls. For the females (Fig. 2) the control curve cuts through the middle of all of the other experimental curves, indicating no effect. Thus, it is evident that those early mouse embryos which



SURVIVAL OF FEMALE MICE: LD/50 X-RAYS FROM 0-5 DAYS POST-FERTILIZATION

FIGURE 2. The dose of x-rays delivered to female mice on gestation days designated varied with the previously established lethal dose to half of the embryos. Here again the most radiosensitive gestational age, with respect to survival, was the embryo exposed at 0.5 day after insemination, with 275 r. However, the deviation from the solid control line was not statistically significant for either male or female mouse. In other words, those mice which survived x-irradiation *in utero* tended to survive as well as did the controls.

TABLE II

Average weights in grams of mice x-rayed in utero during early development

Day x-rayed LD/50	Average weight in grams			
	2 months	12 months	18 months	24 months
males	29.8	37.2	36.1	33.8
0.0 days females	23.9	28.3	31.2	30.8
males	29.1	35.2	33.8	0
0.5 days females	23.3	28.6	29.2	26.4
males	29.4	37.5	37.9	34.8
1.0 days females	22.7	30.7	32.8	29.8
males	30.8	40.2	37.1	33.4
2.0 days females	23.2	29.1	32.7	26.0
males	29.5	38.6	36.4	34.7
3.0 days females	24.3	29.2	32.2	29.9
males	29.7	36.6	35.8	34.5
4.0 days females	23.3	30.2	33.6	33.7
males	29.0	35.1	32.7	30.4
5.0 days females	23.5	28.3	29.2	25.9
males	27.8	36.2	36.3	34.5
Controls females	22.4	28.6	30.7	29.8

tolerate the x-ray exposures used, and survive at birth, and the first month of life thereafter, can be expected to survive almost as well as do the parallel controls.

The monthly weight records for each mouse are reduced to four periods (2, 12, 18 and 24 months) in Table II below. It can be seen that without exception, among the controls or the x-irradiated, the females of the same age were lighter in body weight than were the parallel males. It is also obvious that there was little, if any, statistical difference between the x-irradiated and the control mice by two months of age. The average weight for the first month of age is generally higher for the experimental mice than for the controls because of the reduced litter size of the experimentals and consequently more growing space for the remaining mice. Thus, again it seems evident that x-irradiation of the pre-implantation mouse embryo, if it survives the first post-natal month, will allow it to be as heavy as the controls.

The mice chosen at one month of age for the long term study were radiographed at two months in order to determine whether there was any evidence of permanent skeletal effects. Fifty male and 50 female controls were simultaneously examined, in the same manner as the various irradiated groups (each comprised of 25 males and 25 females). By direct comparison of such averages any contrast with the controls is obvious.

The mice were not anesthetized but were fastened to a plastic board by means of adhesive tape, and were radiographed at 40 volts, 10 MA, at 20 inches distance from

TABLE III

Skeletal measurements by radiography at 2 months of age (in cm.)

Gest. day	r	Sex	Tot. #	Skull		Spine	Humerus	Ulna	Femur	Tibia
				Lat.	A.P.					
Controls	0	M	50	1.05	1.44	6.11	1.20	1.39	1.47	1.68
		F	50	1.04	1.42	5.90	1.15	1.35	1.46	1.67
0.0	100	M	25	1.08	1.50	6.45	1.23	1.42	1.52	1.72
		F	25	1.09	1.46	6.08	1.19	1.38	1.49	1.71
0.5	275	M	25	1.08	1.47	6.21	1.21	1.40	1.51	1.70
		F	25	1.07	1.43	6.01	1.16	1.35	1.48	1.66
1.0	350	M	25	1.07	1.46	6.25	1.20	1.38	1.50	1.69
		F	24	1.05	1.44	5.95	1.14	1.35	1.46	1.68
2.0	125	M	25	1.10	1.50	6.22	1.22	1.42	1.53	1.74
		F	24	1.08	1.46	6.05	1.18	1.37	1.49	1.69
3.0	140	M	25	1.07	1.47	6.49	1.26	1.43	1.53	1.74
		F	25	1.06	1.44	6.28	1.20	1.39	1.52	1.71
4.0	350	M	23	1.05	1.46	6.35	1.23	1.40	1.51	1.71
		F	25	1.04	1.46	6.18	1.20	1.40	1.53	1.73
5.0	350	M	25	1.07	1.47	6.36	1.23	1.42	1.51	1.72
		F	24	1.06	1.45	6.23	1.20	1.39	1.52	1.74

the x-ray source, for two seconds, over sheet film. This amount of x-irradiation was regarded as inconsequential at two months of age. The radiographs were of sufficient clarity as to allow exact measurements of the skull (lateral and AP); spine, humerus, ulna, femur and tibia. Since the measurements were taken from a minimum of 23 x-irradiated mice, and 50 controls of each sex, giving a total of 345 experimental and 100 control radiographs, the data have statistical validity. Table III below gives the average measurement in centimeters for both sexes of each group of experimental and control animals.

It can be seen that the early x-irradiation of the mouse embryo had no adverse effect on the skeletal growth of the survivors, and in fact there appeared to be a tendency of those x-irradiated to be slightly larger than the controls. Again, it

must be pointed out that x-irradiated mice came from depleted litters so that the survivors had more room in which to grow, hence at birth and at one or two months they would be expected to be heavier and have larger skeletal parts than did the parallel controls. It was found by Rugh, Duhamel, Osborne and Varma in 1964 that mice x-rayed to 100 r at 12 to 14 days gestation showed serious defects in skeletal growth, and were all somewhat stunted, but these pre-implantation embryos, x-rayed from 100 r to 350 r, were unaffected with respect to the ultimate skeletal size.

Complete blood counts were made of both the control and x-rayed mice at 2, 6, 9, 12, 18 and 24 months of age. This included the usual determination of hemoglobin, white and red cell counts, platelets, and differentials based on 100 W.B.C.'s. The data do not deviate sufficiently from the controls to be presented in detail; suffice it to say that the x-irradiated mice tended to have slightly higher white cell counts and slightly lower red cell counts than the parallel controls. Whatever damage may have been produced in the mice from x-irradiation during the first five days of gestation was rectified by the time the survivors were two months of age.

As all mice progressed in age there was a drop in the hemoglobin and erythrocyte counts. Similarly a general trend was observed in the decrease of the number of lymphocytes and an increase in the neutrophils from two to 24 months of age. There was no evidence of leukemia in any of the mice examined. At 24 months four cases of lymphocytosis were found, one of which was a control. The leukocyte counts varied from 45,000 to 163,000 in these mice, of which an average of 89% were lymphocytes, all of a mature type. No further histological examination was provided for so that the exact nature of the lymphocytosis could not be determined. At 18 months the highest leukocyte count was 21,800 with no comparable lymphocytosis being noted.

A total of 1098 examinations of mice are presented for cataracts (2176 eyes) at 2, 12, 18 and 24 months of age. Examinations were also made at 6, 9 and 29 months but these data are not included in Table IV. Three major effects were recorded: corneal opacities, which are apparently minor abrasions of the cornea (conjunctiva) from which many eyes recover; nuclear sclerosis, which is a pre-cataract condition; and the distinct cataract. The corneal opacities seemed to be unrelated to the onset and development of cataracts while nuclear or even cortical sclerotic conditions of the lens almost always led to cataracts. Occasionally a corneal opacity obscured a lens in such a manner as to make the determination of a cataract difficult.

For the controls there were 50 males and 50 females at the beginning of the study, when the mice were one month of age. For each of the groups of mice x-irradiated at the various gestation days there were selected 25 males and 25 females, also at one month of age. These original numbers dropped steadily after 12 months of age so that by 24 months there were very few mice alive, either x-rayed or controls (Figs. 1 and 2). Thus the cataract data are derived as percentage data rather than actual numbers, and such percentages become less significant as the total number decreases (Table IV).

The incidence of cataracts at 18 months for the controls corresponds very well with the data of the previous study by Rugh, Duhamel, Chandler and Varma in 1964, but by 24 months even the controls showed an increasing incidence of

TABLE IV*

Cataract incidence following LD/50 x-ray exposure of mouse embryos fertilization to 5 days

	2 months					12 months					18 months					24 months				
	# Mice	% CO	% Scl.	% Cat.	% Tot. Cat.	# Mice	% CO	% Scl.	% Cat.	% Tot. Cat.	# Mice	% CO	% Scl.	% Cat.	% Tot. Cat.	# Mice	% CO	% Scl.	% Cat.	% Tot. Cat.
Controls	♂50	44	0	0	0	38	43	1	5	6	26	38	1.9	12	14	13	38	8	31	39
	♀50	50	0	0	0	41	45	9	6	15	27	50	7	7	14	13	27	12	54	66
0.0 day	♂25	50	2	0	2	21	17	0	36	36	14	14	4	39	43	7	21	0	36	36
(100 r)	♀25	52	0	0	0	21	21	5	33	38	13	19	8	54	62	5	20	0	100	100
0.5 day	♂25	24	0	0	0	14	4	7	0	7	5	10	20	0	20	0	0	0	0	0
(275 r)	♀25	56	0	0	0	22	2	2	20	22	11	5	0	41	41	2	25	0	25	25
1.0 day	♂25	66	0	0	0	22	7	0	18	18	8	31	0	19	19	3	0	0	50	50
(350 r)	♀24	67	0	0	0	20	5	2.5	15	18	11	5	27	14	41	2	0	0	0	0
2.0 days	♂25	56	0	0	0	20	50	5	5	10	11	45	27	18	45	2	50	0	50	50
(125 r)	♀24	54	0	0	0	21	45	5	14	19	11	45	9	14	23	4	50	0	25	25
3.0 days	♂25	50	0	0	0	23	20	4	15	19	5	20	20	20	40	2	0	0	50	50
(140 r)	♀25	56	0	0	0	21	10	2	19	21	13	4	0	8	8	5	0	30	40	70
4.0 days	♂24	58	0	0	0	16	34	0	6	6	10	35	10	10	20	2	0	0	100	100
(330 r)	♀25	46	0	0	0	24	15	17	4	21	12	29	17	17	34	5	0	2	60	80
5.0 days	♂25	32	0	0	0	18	28	0	14	14	11	32	9	14	23	3	0	0	66	66
(350 r)	♀24	40	2	0	2	21	21	7	19	26	13	8	19	23	32	9	6	6	56	62

* CO = corneal opacity, which was generally transient; Scl. = sclerosis, generally nuclear but occasionally cortical; Cat. = distinct cataract. Note that survivors at 18 months showed a higher incidence of cataracts among those x-rayed *in utero* than in the controls, but that at 24 months the data are irregular, because of the high mortality intervening.

cataracts (males 39% and females 66%). This suggests that such cataracts are truly senile cataracts, but the onset in this strain of mice appears to be slower than in some other strains.

It appears that homozygous mice can have congenital cataracts, while heterozygous mice tend to have normal vision. The onset of cataractogenesis in different strains may differ considerably. It is of interest to note that within any group of similar mice, similarly irradiated, cataract development is never all-or-none; there is a great variation in response (Gowen, 1962).

Among the x-irradiated mice surviving to 18 months, in almost every set of data it is obvious that those x-rayed had a higher incidence of cataracts than did the controls at the same age. However, when the incidence of cataracts among the few survivors at 24 months is determined, the range was from 0% to 100% among those x-rayed, as compared with an average of 53% for the controls. It must be recalled that mortality of many irradiated mice left only the most hardy ones to be included in this study.

Summary and Conclusions

1. Either x-rayed or control male mice had average weights in excess of the females at a comparable age. Pre-implantation mouse embryos, subjected to x-rays and surviving for 24 months, showed no gross adverse weight effects of the exposures. In some instances those with a radiation history were heavier, probably because they came from depleted litters which had more growing space within the uteri.

2. Whole blood counts indicated that mice x-irradiated in the pre-implantation stage tended to have slightly higher white cell counts and slightly lower red cell counts than their parallel controls. Otherwise any possible hematological damage appears to have been rectified.

3. There were no permanent skeletal effects on mice x-irradiated *in utero* during the pre-implantation stages of 0.0 to 5.0 days, as determined by radiographs of five selected bones and two skull measurements at 2 months of age.

4. Mice x-rayed at fertilization or at 5 days gestation showed almost as good survival as did the controls, but those x-rayed on days 1, 2, 3 and 4 showed slightly reduced survivals.

5. Corneal opacities occur frequently in these mice. Their eyes appear to be anesthetized to the particulate material in the bedding. There appeared to be no direct relationship between corneal opacities and the development of cataracts. Many mice with corneal opacities at two months recovered normal corneas at a later date.

6. Mouse cataracts appear to arise as nuclear or cortical sclerosis of the lens, but those arising in the nuclear region appear to be in the majority. The ultimate cataract, regardless of its origin, appeared to be similar in its involvement.

7. At any test period the percentage incidence of cataracts among the survivors was always higher among those x-rayed *in utero* than among the parallel controls.

8. Variations in cataractogenesis existed between males and females similarly x-rayed, as had also been shown in the previous study with the uniform exposure of 100 r x-rays. There appeared to be a sex differential in cataractogenesis of x-ray origin.

9. The fact that cataracts appeared earlier and to a greater extent among the x-irradiated mice than among the controls suggests that x-rays may hasten the onset of the usual senile cataracts.

10. There appears to be a greater incidence of bilateral as opposed to unilateral cataracts, and this seems to be particularly true for the females. The incidence of cataracts in one eye leading to bilateral cataracts occurred more frequently in mice x-rayed at fertilization and in females x-rayed at 1.0 and 5.0 days gestation. Thus, there was no clear-cut evidence of uni- leading to bilateral cataract development except possibly among some potential females. The precursors of the two eyes of any mouse at these early stages presumably received the same degree of radiation insult.

11. Since this study is based entirely upon x-irradiation of the early mouse embryo from fertilization to 5 days gestation, and since it has been demonstrated that x-rays during this period do in fact increase the incidence of cataracts, it must be presumed that the damage is done to the precursors of the lens since lens development is not initiated until about 11 days gestation. It is suggested that the etiology of these radiation-induced cataracts may be through an interference with the developmental process, originating with damage to chromosomes insufficient to be lethal.

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